Manual Sample Loading Techniques for Centri•Sep 96 filter plates

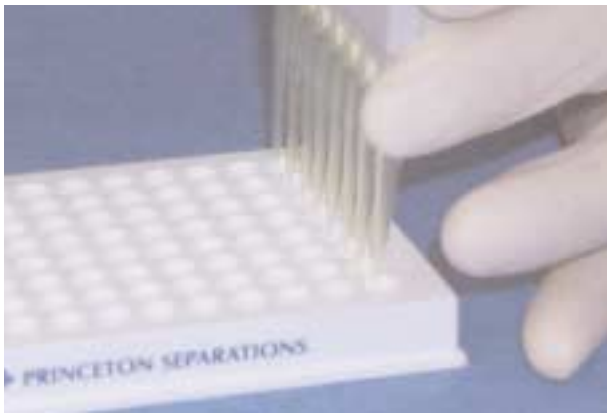
T-4

Centri•Sep 96 filter plates have been developed and optimized to remove the excess dye terminators from the extension products in the Big Dye™ Terminator cycle sequencing reactions. Sample volumes of 5 to 20 microliters are efficiently purified in less than 10 minutes. Purified samples result in clean electrophoretic results and long base reads. Transferring the samples to the plates is an extremely important part of the procedure and is essential to getting good quality sequencing results.

Centri•Sep 96 filter plates are manufactured using precision filling equipment. The gel bed heights are extremely uniform in all of the wells on a plate and automated liquid transfer instruments or robots are recommended for sample loading whenever possible. If manual loading is necessary, the following points should be considered.

- Samples must be added to the center of the resin bed without disturbing the gel surface.
- Avoid contact between the pipette tip and the side of the well. Excess reaction mixture on the tip may 'wick' off onto the side of the well. This can result in a dye front on the sequencing gel.
- When using 8 channel pipettors (12 channel are not recommended), hold the pipettor between the thumb and forefinger of one hand and rest this hand on the plate surface (see Figure 1). Guide the pipettor so that the tips are perpendicular to the surface of the wells, directly over the center of the resin plugs and about 2mm above the plug surface.
- Slowly depress the plunger with the other hand so that the sample flows onto the resin bed without disturbing the surface. Expelling the sample too quickly will force the liquid deep into the bed resulting in an incomplete removal of the dye. It may help to practice with only 4 tips attached to the pipettor.
- Perform routine maintainence on the pipettor per the manufacturer's directions. Pay particular attention to the action of the plunger. It must move smoothly through its entire travel without sticking.
- Sample loaders based on multi-syringe formats have consistently provided excellent results. This may be due to the reduced velocity of the sample when expelled from the loader.

This technique



Not this

Figure 1. Pipettor Orientation

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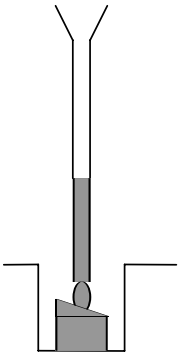
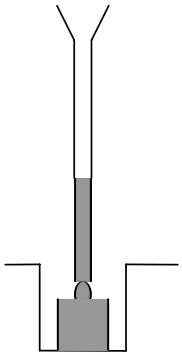
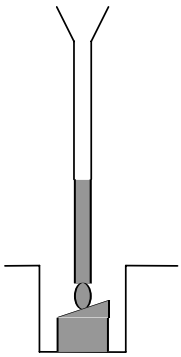
Correct Method for Sample Loading



End Wells



Center Wells

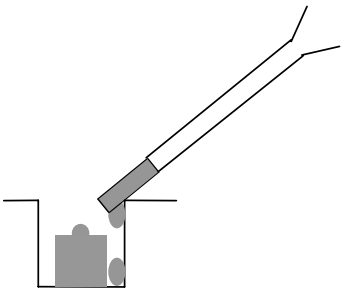


End Wells

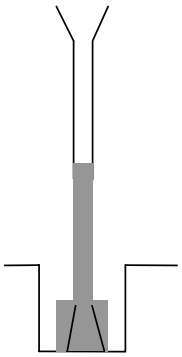
Incorrect Methods for Sample Loading

DO NOT

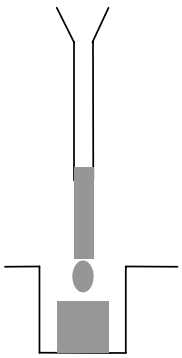
- Touch the sides of the wells with pipette tips or small drops of sample.
- Angle the tips into the wells. (Position tips vertically 2mm above the surface.)
- Push the tips into the gel bed.
- Hold the tips high above the plate and allow the sample to drop onto the surface of the gel bed.
- Depress the plunger rapidly, forcing sample into the gel bed.



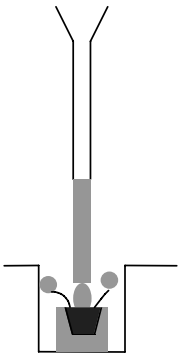
Tips angled and touching side of well



Tips are touching gel surface



Tips too high above surface



Erratic piston stroke.

Note: In addition to the correct tip positioning, a smooth piston stroke on the pipettor is required. A fast and/or erratic piston stroke will cause the sample to splash to the sides of the wells or force the sample too far into the resin bed.

Remember:

Poor Sample Transfer may result in Dye carry-over onto Sequencing Gel

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